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## **Effect of age on cutaneous vasomotor responses during local skin heating.**

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## Abstract

This study examined the effect of ageing on the low-frequency oscillations (vasomotion) of skin blood flow in response to local heating (LH). Skin blood flow was assessed by laser-Doppler flowmetry on the forearm at rest (33°C) and in response to LH of the skin to both 42°C and 44°C in 14 young (24±1 years) and 14 older (64±1 years) participants.

Vasomotion was analysed using a wavelet transform to investigate power of the frequency intervals associated with endothelial, neural, myogenic, respiratory, and cardiac activities of the laser-Doppler signal. Laser-Doppler flux increased in both groups with LH (both  $d > 1.8$ ,  $p < 0.001$ ). Endothelial activity increased in both groups following LH to 42°C (young  $d = 1.4$ ,  $p < 0.001$ ; older  $d = 1.2$ ,  $p = 0.005$ ) and 44°C (young  $d = 1.4$ ,  $p = 0.001$ ; older  $d = 1.5$ ,  $p = 0.005$ ). Endothelial activity was higher in the young compared to older group during LH to 42°C ( $d = 1.4$ ,  $p = 0.017$ ) and 44°C ( $d = 1.5$ ,  $p = 0.004$ ). In response to LH to 42°C and 44°C, neural activity in both groups was decreased (both groups and conditions:  $d > 1.2$ ,  $p < 0.001$ ). Myogenic activity increased in the younger group following LH to 44°C ( $d = 1$ ,  $p = 0.042$ ), while in the older group, myogenic activity increased following LH to 42°C ( $d = 1.2$ ,  $p = 0.041$ ) and 44°C ( $d = 1.1$ ,  $p = 0.041$ ). Respiratory and cardiac activities increased in both groups during LH to 42°C and 44°C (All:  $d > 0.9$ ,  $p < 0.017$ ). There were no differences in wavelet amplitude between younger and older in the neural ( $d = 0.1$ ,  $p > 0.7$ ), myogenic ( $d = 0.3$ ,  $p > 0.7$ ), respiratory ( $d = 0.4$ ,  $p > 0.6$ ), and cardiac ( $d = 0.1$ ,  $p > 0.7$ ) frequency intervals. These data indicate that LH increases cutaneous endothelial and myogenic activity, while decreasing neural activity. Furthermore, ageing reduces the increase in cutaneous endothelial activity in response to LH.

**Keywords:** skin blood flow, wavelet analysis, ageing, endothelial, spectral analysis

### **Highlights**

- We examined the effect of ageing on cutaneous vasomotion to local skin heating
- Laser-Doppler and spectral analysis was performed on 14 young and 14 older humans
- Cutaneous endothelial and myogenic activities increase and neural activity decreases
- Endothelial activity was greater in the young compared to the older group
- Age-related changes in endothelial function can be examined non-invasively

## **Introduction**

Two strengths of laser-Doppler flowmetry are that it is non-invasive and provides a continuous measurement. The majority of studies examining the mechanistic control of the cutaneous vasculature have used needle-based invasive techniques (e.g. intradermal microdialysis) to administer pharmacological agents (Johnson et al., 2014). While artifacts associated with needle trauma can be limited by temporary anaesthesia (Hodges et al., 2009a), it is clear that implantation affects vasodilator function (Hodges et al., 2009a), and there is the chance that the invasive procedures affect vascular function more than realized (Groth, 1998; Groth et al., 1998; Groth and Serup, 1998; Sjogren and Anderson, 2009).

Examination of low-frequency oscillations inherent to the skin blood flow signal enables a non-invasive assessment of regulatory mechanisms of the cutaneous circulation (Bracic and Stefanovska, 1998; Rossi et al., 2006; Rossi et al., 2008; Stefanovska et al., 1999). Strengths of this approach compared to other non-invasive or pharmacological-dependent methods of assessing mechanistic control of the vasculature is that, unlike a procedure such as flow-mediated dilatation, there is no need for a trained sonographer or expensive edge-tracking software. Furthermore, wavelet analysis can be used for microcirculatory function and provide information on not only endothelial activity, but also, neural, myogenic, respiratory and cardiac influences (Bracic and Stefanovska, 1998; Kvandal et al., 2006; Kvandal et al., 2003; Soderstrom et al., 2003). Indeed, characteristic peaks have been identified within the vasomotion signal; ranging from the cardiac and respiratory rhythms at approximately 1.6 Hz and 0.6 Hz respectively. Endothelium-related oscillations of approximately 0.01 Hz have been shown via applying both agonists and antagonists (Kvandal et al., 2006; Kvandal et al., 2003; Rossi et al., 2006; Rossi et al., 2008; Stefanovska et al., 1999). Furthermore, the neural influence on skin blood flow has been shown to be associated with a frequency range of 0.02–0.05 Hz (Soderstrom et al., 2003). Thus, wavelet analysis can provide a considerable amount of mechanistic information regarding microvascular function.

Ageing has been reported to decrease both the initial peak (Minson et al., 2002; Tew et al., 2011a; Tew et al., 2011b), and the sustained vasodilatation to prolonged local skin heating (Hodges et al., 2010a; Hodges et al., 2010b; Martin et al., 1995; Minson et al., 2002; Tew et al., 2012a). Evidence indicates that the age-related reduction in the initial peak response is due to impaired neural mechanisms, while the impaired plateau phase is due to reduced endothelial activity (Tew et al., 2012b). Indeed, work in other vascular beds demonstrates reduced endothelial activity with increasing age (Green et al., 2011; Thijssen et al., 2010). To date, no study has examined the effect of ageing on the cutaneous vasomotor response to local skin heating.

Thus, wavelet analysis of the laser-Doppler signal offers the opportunity to develop a relatively short, painless, non-invasive procedure capable of providing mechanistic insight to microcirculatory function. Not only can flux and reactivity responses be measured, but endothelial, neural, myogenic, respiratory, and cardiac mechanisms can also be assessed. If this approach can be shown to be sensitive enough to examine the effects of ageing and disease processes affecting microvascular function (Podtaev et al., 2015), this would be a highly useful clinical tool in diagnosing gross microvascular (dys)function, as well as characterising the endothelial and neural changes that accompany ageing.

Therefore, we sought to determine the effect of ageing on the low-frequency oscillations in cutaneous blood flow at rest and during local skin heating. We hypothesized that local heating would increase endothelial activity in both younger and older individuals, with the increase being greater in the younger compared to the older individuals. We also hypothesized that neural activity during the initial peak phase would be increased in the younger group versus the older group, but that neural activity would be reduced during the local skin heating to 42 °C and 44 °C in both groups

## **Methods**

### *Ethical approval*

This study was approved by the local research ethics committee at Sheffield Hallam University. All volunteers provided written, informed consent prior to participation. This study was carried out in accordance with the Declaration of Helsinki.

### *Participants*

Power analysis ( $\alpha$  of 0.05 and  $\beta$  of 0.20) determined that 12 participants were needed to determine differences in vasomotion between age groups. Fourteen young and 14 older men without cardiovascular disease, hypertension, diabetes, or cancer, who were not smokers or taking any form of medication were recruited. Each participant visited the laboratory on two occasions. For both sessions, they were asked to refrain from caffeine, alcohol, and exercise for 24 h prior. The physical characteristics of the two groups are presented in Table 1.

### *Visit 1: Cardiopulmonary fitness assessment*

All participants performed a continuous, incremental cycling test to volitional exhaustion on an electronically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands). Pedalling frequency was self-selected within 60 to 90 rpm. Following a 2 min warm-up at 0 W, resistance was increased by 20-30 W  $\cdot$  min<sup>-1</sup>, with participants continuing until volitional exhaustion or a plateau in oxygen uptake. Heart rate was collected via ECG. The volume of oxygen consumed during exercise was calculated from minute ventilation, measured using a pneumotachometer, and simultaneous breath-by-breath analysis of expired gas fractions (Ultima CardioO<sub>2</sub>; MedGraphics, St. Paul, MN, USA). Gas analysers and flow probes were calibrated before each test. Oxygen uptake was expressed relative to body mass (ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>). Maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) was calculated as the highest 20 s period of gas exchange data in the last minute before the end of the test.

### *Visit 2: Microvascular assessment*

#### *Instrumentation and experimental procedure*

Experiments were performed in a temperature-controlled room (22-24 °C), with participants resting supine and the experimental arm (left) positioned at heart level for the entire protocol. A site on the ventral aspect of the left forearm was chosen, avoiding visible veins, damaged or irritated skin, and hair. Skin blood flow was measured as cutaneous red blood cell flux using a laser-Doppler fluxmeter (LDF; Periflux system 5000, Perimed AB, Järfälla, Sweden) and a 7-point integrating LDF probe (Probe 413, Perimed AB). Local skin heating was performed using a heating disc surrounding the probe (Model 455, Perimed AB), connected to a heating unit (Model 5020, Perimed AB). Recordings of the laser-Doppler signal were made using PeriSoft for Windows 9.0 software (PSW 9.0, Perimed AB). Blood pressure was measured using an automated blood pressure cuff every 2 min (Dinamap Dash 2500; GE Healthcare, Waukesha, WI, USA).

#### *Wavelet transform*

A Morlet mother wavelet was used to perform wavelet analysis on the LDF signal. Wavelet analysis was chosen as it provides good time and frequency resolution within the uncertainty principle. It uses an adjustable window to provide good frequency resolution for lower and higher frequencies by using a longer and shorter analysis window, respectively. This method characterizes the dynamics of signals over a wide frequency range, from 0.0095 to 1.6 Hz.

Analysis of time segments of 20 min is needed for good low-frequency resolution and detection of oscillations in the low-frequency range (Stefanovska et al., 1999). We ran the wavelet transform on a minimum of 55 min of data. While we have chosen short time windows for certain phases to extract median amplitudes of the vasodilator responses (initial peak and nadir), the fact that the wavelet was run on the entire data laser-Doppler signal ensured that even these smaller extracted portions had appropriate resolution and power to examine the low-frequency bands.

#### *Data collection and statistical analysis*

LDF data were collected at 32 Hz (PSW 9.0, Perimed AB). The data from the entire protocol ~60 min was exported and run through the wavelet transform in a custom written



computer script (Iatsenko et al., 2013; Iatsenko et al., 2015; Iatsenko et al., 2016) (Matlab®, The MathWorks Inc., Natick, MA, USA). Subsequently, median amplitudes were chosen from the appropriate place (Fig. 1) such that data for basal (4 min), initial peak (30 s), nadir (30 s), plateau at 42 °C (4 min), and maximum at 44 °C (4 min) were extracted.

Vasomotion data (AU) were not normally distributed as assessed by the Shapiro-Wilk test of normality. A Friedman one-way ANOVA was used to examine local heating responses (baseline, initial peak, nadir, plateau, max) for each group. Dunn's correction was used for multiple comparisons. A Kruskal-Wallis test was used for the vasomotion analysis between groups and Dunn's correction for multiple comparisons was used. Data are presented as box plots, with median, quartiles, min, and max represented (v6.01, GraphPad Software Inc., La Jolla, CA, USA). In the text, data are presented as median and interquartile range. Cohen's *d* effect sizes and 95% confidence intervals [95%CI] were calculated for the differences between heating phases and groups sizes (ESCI-delta, Geoff Cumming, La Trobe University, AU) and interpreted using the following classification:  $d = 0.20-0.49$  = small effect;  $d = 0.50-0.79$  = moderate effect and  $d > 0.8$  = large effect (Cohen, 1988).

Laser-Doppler data (PU) were normally distributed as assessed by the same criteria above. A repeated measures one-way ANOVA was used to examine heating responses within groups with a Bonferroni *post-hoc* correction for multiple comparisons. Group differences were analysed using a one-way ANOVA with a Bonferroni correction for multiple comparisons. Data are presented as mean and SD. Effect sizes were calculated using Cohen's *d*. Statistical analyses were performed using GraphPad Prism (v6.01, GraphPad Software Inc., La Jolla, CA, USA) and SAS v9.13 (SAS institute Inc., Cary, NC, USA) where statistical significance was defined as  $P < 0.05$ .

## Results

### *Participant characteristics*

As specifically recruited, the older group were significantly older than the younger group ( $p < 0.05$ ) (Table 1). While all participants were normotensive, the older group had statistically greater resting diastolic blood pressure compared to the younger group ( $p < 0.05$ ); there was no difference in systolic blood pressure or heart rate. Pulse pressure was greater ( $p < 0.05$ ) in the younger group compared to the older group. Additionally,  $\dot{V}O_{2\max}$  was greater in the younger group compared to the older group ( $p < 0.05$ ). There were no differences in stature and body mass.

### *Laser-Doppler flux*

Mean LDF data for all participants are presented in Figure 2. Local skin heating caused an increase in flux in both groups ( $d \geq 4.7$ ,  $p < 0.05$ ). There was no statistical difference in the vasodilator response between the young and old groups at any of the phases (all  $d \leq 0.6$ ,  $p > 0.05$ ).

### *Wavelet Analysis*

*Endothelial (0.0095 – 0.021 Hz)* Under thermoneutral (33 °C) conditions, there were no statistical differences in wavelet amplitude between the young and older groups ( $d = 0.9$  [0.2 1.4];  $p > 0.05$ ) (Fig. 3). After increasing local skin temperature to 42 °C, there were also no statistical differences in wavelet amplitude of the initial peak ( $d = 0.6$  [0.2 1.3];  $p > 0.05$ ) and nadir ( $d = 0.3$  [0.1 1.0];  $p > 0.05$ ) phases of the cutaneous vasodilator response. However, after  $\geq 30$  min of maintained local skin temperature at 42 °C, wavelet amplitude was greater in the young group compared to the older group ( $1.715 \pm 0.712$  AU vs.  $0.955 \pm 0.303$  AU) ( $d = 1.9$  [1.0 2.8];  $p < 0.05$ ). The younger group also had greater wavelet amplitude ( $1.749 \pm 0.534$  AU) after increasing local skin temperature to 44 °C for 25 min compared to the older group ( $0.873 \pm 0.316$  AU) ( $d = 2.2$  [1.2 3.1];  $p < 0.05$ ).

*Neural (0.021 – 0.052 Hz)* Compared to thermoneutral baseline (33 °C), local skin heating to 42 °C for  $\geq 30$  min decreased the wavelet amplitude in both the young ( $d \geq 1.4$  [0.6 2.2];  $p < 0.05$ ) and older ( $d = 2.1$  [1.1 3.0];  $p < 0.05$ ) groups (Fig. 4). Wavelet amplitude did not change

from baseline during the other phases of the vasodilator response (all  $d < 0.5$ ,  $p > 0.6$ ). There were no differences between the young and older groups at baseline ( $d = 0.4$  [0.1 1.2];  $p > 0.05$ ), initial peak ( $d = 0.5$  [0.1 1.3];  $p > 0.05$ ), nadir ( $d = 0.5$  [0.1 1.2];  $p > 0.05$ ), plateau ( $d = 0.2$  [0.1 0.8];  $p > 0.05$ ), and max ( $d = 0.4$  [0.1 1.1];  $p > 0.05$ ).

*Myogenic (0.052 – 0.145 Hz)* Local skin heating to 42 °C and 44 °C increased wavelet amplitude in the young group compared to baseline (33 °C) ( $d = 0.7$  [0.1 1.5];  $p < 0.05$  and  $d = 1.1$  [0.3 1.9];  $p < 0.05$ ) (Fig. 5). In the older group, heating to 42 °C increased wavelet amplitude in this frequency band during the plateau and maximum phases compared to baseline and nadir phases ( $d \geq 1.1$  [0.4 1.8];  $p < 0.05$ ). There were no differences between the young and older groups at baseline ( $d = 0.5$  [0.1 1.3];  $p > 0.05$ ), initial peak ( $d = 0.5$  [0.2 0.8],  $p > 0.05$ ), nadir ( $d = 0.2$  [0.1 0.9],  $p > 0.05$ ), plateau ( $d = 0.3$  [0.1 1.0],  $p > 0.05$ ), and max ( $d = 0.7$  [0.1 1.5],  $p > 0.05$ ).

*Respiratory (0.145 – 0.6 Hz)* In the younger group, wavelet amplitude compared to baseline ( $0.746 \pm 0.388$  AU) was increased during the local heating to 42 °C ( $1.186 \pm 0.592$  AU;  $d = 1.0$  [0.2 1.8];  $p < 0.05$ ) and 44 °C ( $1.352 \pm 0.704$  AU;  $d = 1.3$  [0.4 2.1];  $p < 0.05$ ). This was the same for the older group, with respiratory amplitude at baseline ( $0.511 \pm 0.23$  AU) increasing in response to local skin heating to 42 °C ( $1.18 \pm 0.668$  AU;  $d = 2.1$  [1.1 3.0];  $p < 0.05$ ) and 44 °C ( $1.348 \pm 0.58$  AU;  $d = 1.8$  [0.9 2.7];  $P < 0.05$ ). The effect sizes between the groups throughout the 5 phases examined were: baseline  $d = 0.8$  [0.1 1.5], initial peak  $d = 0.1$  [0.1 0.8], nadir  $d = 0.2$  [0.5 0.9], plateau  $d = 0.4$  [0.1 1.1], max  $d = 0.3$  [0.1 1.0]. There were no statistical difference between the groups at any of the phases (all  $p > 0.05$ ).

*Cardiac (0.6 – 1.6 Hz)* Cardiac amplitude increased in the young from baseline ( $0.702 \pm 0.431$  AU) with local skin heating to 42 °C ( $1.729 \pm 0.706$  AU;  $d = 1.9$  [1.0 2.9];  $p < 0.05$ ) and 44 °C ( $1.976 \pm 1.07$  AU;  $d = 2.2$  [1.3 3.2];  $p < 0.05$ ). This was also the case for the older group with significant increases during the heating from 33 °C ( $0.435 \pm 0.277$  AU) to 42 °C ( $1.563 \pm 0.892$  AU;  $d = 2.2$  [1.2 3.1];  $p < 0.05$ ) and max ( $1.806 \pm 0.919$  AU;  $d = 2.3$  [1.3 3.2];  $p < 0.05$ ) phases. There were no differences in the local heating response (all phases  $d = 0.1$  to  $0.7$ ,  $p > 0.5$ ) between the groups.

## Discussion

The aim of this study was to examine the effect of age on cutaneous vasomotion responses in rest, and in response to local skin heating, in healthy young and older adults. We found that local skin heating increased low frequency oscillations associated with endothelial, myogenic, respiratory, and cardiac activity while neural activity was decreased.

Additionally, we found that the younger group had a greater increase in endothelial activity in response to local skin heating than the older group. Finally, there were no differences between the young and older groups for any of the other frequency bands analyzed. These data show that that vasomotion analysis could be a useful non-invasive tool for mechanistic studies of microvascular function in ageing and disease.

Previous work, using the invasive technique of intradermal microdialysis to deliver pharmacological interventions established that the cutaneous vasodilator response to local skin heating is mediated by the endothelium (Brunt and Minson, 2012; Kellogg et al., 1999; Kellogg et al., 2008a). Inhibition of endothelial nitric oxide synthase prior to (Kellogg et al., 2008a) or after (Hodges and Sparks, 2014) local skin heating causes a robust decrease in local heating-induced vasodilatation. Similarly, inhibition of endothelial dependent hyperpolarizing factors also reduced the vasodilatation attendant to local skin heating (Brunt and Minson, 2012). Using this non-invasive approach, we did not observe any differences in endothelial function between younger and older groups during basal measurements; this is consistent with previous invasive work that has demonstrated no effect of nitric oxide synthase inhibition on basal skin blood flow (Kellogg et al., 2008a; Kellogg et al., 2008b; Kellogg et al., 2009). We did however, find reduced endothelial activity in the older group compared to the younger group after prolonged heating (Fig. 3). This is the first study to directly examine the effects of primary ageing on cutaneous endothelial activity in response to local skin heating. This finding is consistent with invasive studies examining the effect of ageing and aerobic exercise training on endothelial function using endothelial agonists (Hodges et al., 2010b) and antagonists (Black et al., 2008). Our results indicate that non-invasive procedures can be used to examine

mechanisms of action, notably, changes in endothelial activity of the cutaneous circulation with sufficient sensitivity to distinguish differences between healthy old and young populations.

The endothelium itself is not the only site influenced by the ageing process that could be contributing to this observed decrement in vasodilatation. Indeed it has been reported that ageing impairs nitric oxide-mediated relaxation of smooth muscle (Utkan et al., 2002). The sites downstream of the production of endothelial-derived vasodilators are also impaired with ageing, consequently reducing vasodilatation regardless of changes in endothelial function. While the present study did not measure vascular smooth muscle function, it would make sense that any age-related changes in the ability of the smooth muscle to utilise endothelial-derived vasodilators would contribute to the observed reduction in skin blood flow of the older cohort to local heating, which is almost exclusively dependent on endothelial vasodilators (Brunt and Minson, 2012; Kellogg et al., 2008a; Kellogg et al., 2009). Given the strong prognostic links between endothelial function and cardiovascular events (Barlow et al., 1995; Blair et al., 1995; Dimmeler and Zeiher, 2003; Xu et al., 2014) the implications of changes in these with ageing are of obvious importance.

Sensory and sympathetic nerve blockade reduce initial peak responses in young and older individuals (Del Pozzi and Hodges, 2015; Hodges et al., 2008; Hodges et al., 2009b; Tew et al., 2011a; Tew et al., 2011b), thus we speculated that neural activity would be increased during this phase; however, this was not observed in either group. Instead, neural activity was decreased during skin heating to 42 °C and 44 °C. We recently showed that antagonizing adrenergic or NPY (cutaneous sympathetic nerve neurotransmitters) after the plateau phase is established, has no effect on the vasodilator response (Hodges and Sparks, 2014). These findings are difficult to explain due to the effects of sympathetic blockade prior to local heating, but indicates that once established, the locally-mediated vasodilator process no longer requires adrenergic or NPY stimulation. Hence, contrary to previous work that indicated impaired cutaneous sympathetic neural activity with ageing, we observed no age-related differences in neural activity between the groups.

Previous studies have reported a progressive age-related decrease in skin blood flow in response to local skin heating using plethysmography (Martin et al., 1995) and laser-Doppler fluximetry (Hodges et al., 2010a). Notably, the present study did not see a difference in laser-Doppler flux between the young and older groups. This might be due to blood pressure differences in the groups ( $\sim 118/66$  vs.  $\sim 122/76$  mm Hg) as the data in those previous studies were present as conductance not flux. Additionally, the large heterogeneity of the cutaneous circulation must be considered in a cross-sectional study such as this. Thus, the large variability in site-to-site vessel density might skew the flux readings using such small areas of assessment (Johnson et al., 1984). The fact that the LDF is similar between the young and older groups might be related to skin thickness. Older people have thinner skin than younger people (Fenske and Lober, 1986), thus maybe the higher flux in the older group was due to more signal returning to the LDF probe due to thin skin. Thicker skin in the young group gives more opportunity for the laser-light to be scattered, and therefore, receive less LDF signal. This likely increases the volume of assessment by the laser-Doppler probe in older people. Indeed, this has been noted in a recent publication (Ticcinelli et al., 2015) examining wavelet transform analysis on resting cutaneous laser-Doppler signal in older and younger populations. They reported higher spectral power in the older group compared to the younger group. Also, they noted that the increased vessel radius and decreased vessel elasticity may have led to a reduced effect of local active oscillations compared to the centrally generated waves (respiratory and cardiac) leading to stronger propagation. This might explain the large increases and apparent influence of these bands during local skin heating. It may also provide insight into why there were such similarities between the groups as the area of assessment might have been larger in the older group relative to the younger group based on penetration depth of the LDF signal, as seen with other tissues.

We noted an increase in myogenic activity of both groups during the prolonged skin heating to 42 °C and 44 °C. This could be due to an increased volume of blood entering the arterioles with smooth muscle relaxation (vasodilatation) in response to local skin heating,

increasing the activity of pre-capillary sphincters, which control the amount of blood entering the capillary bed. Even though the young group had lower blood pressure than the old group, there was little difference in pulse pressure between groups (both ~50 mm Hg). As muscle cells responding to changes in intravascular pressure lead to myogenic activity (Stefanovska, 1999), it makes sense that these groups are not different.

Curiously, we have observed a larger pulse pressure in our younger group compared to our old group. This observation is atypical, as age-related increases in blood pressure are usually attributable to an increase in systolic pressure while there is little change or a slight decrease in diastolic blood pressure (Franklin, 2006a; Franklin, 2006b). This leads to a widening in pulse pressure in older individuals. In our cohorts, we observed no difference in systolic pressure but the older group did have a higher diastolic pressure, despite it still being within the healthy range. Perhaps we have not observed the typical response in our groups, as the participants were supine for their blood pressure measurements. This likely increased venous return to the heart in both groups, preload, and ultimately stroke volume in both groups, however, younger participants typically have more compliant ventricle and aortic walls (Lakatta and Levy, 2003a; Lakatta and Levy, 2003b) allowing for greater filling and this could be leading to larger increases in stroke volume and thus the increased pulse pressure compared to the older participants. Importantly, all participants were normotensive. It should also be noted, that based on normative data compiled by the American College of Sports Medicine (ACSM), that the aerobic capacity of both groups were comparable to norms for their respective age ranges (ACSM, 2000) and the values achieved indicate that both groups were reasonably active.

The true strength of this technique is the ability to obtain mechanistic insight in clinical populations which would otherwise not be possible or difficult to do with invasive or pharmacological produces. Some work has been performed in morbidly obese patients (Rossi et al., 2011) and those with diabetes (Rossi et al., 2013), highlighting that this approach can work in patient populations. Use of vasomotion analyses also reduces the time burden on the patient. Microdialysis and iontophoresis are powerful tools to examine microvascular function, but the former requires ~90 min of trauma resolution from the

fibre placement (Hodges et al., 2009a), and both require time for the effects of the pharmacological agents to work and the vascular response to subside prior to measurements being taken.

In summary, we found that in response to local skin heating there is a marked increase in cutaneous endothelial activity in both young and older healthy adults. The magnitude of this response is smaller in older individuals compared to younger. There were no differences between younger and older individuals in neural, myogenic, respiratory, and cardiac activity in response to local skin heating. These data demonstrate that non-invasive procedures can be used to examine mechanisms (e.g. endothelial function) in the cutaneous circulation and do so with sufficient sensitivity to differentiate the responses of these mechanisms between younger and older individuals.

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### **Declaration of interest**

No conflicts of interest, financial or otherwise, are declared by the authors.

### **Author Contributions**

GJH conceived this project. GAT, MK, JM, ADR undertook the assessment sessions. GJH and MMM analyzed and interpreted the data. All authors provided intellectual input, as well as contributing to and approving the final version of this manuscript.

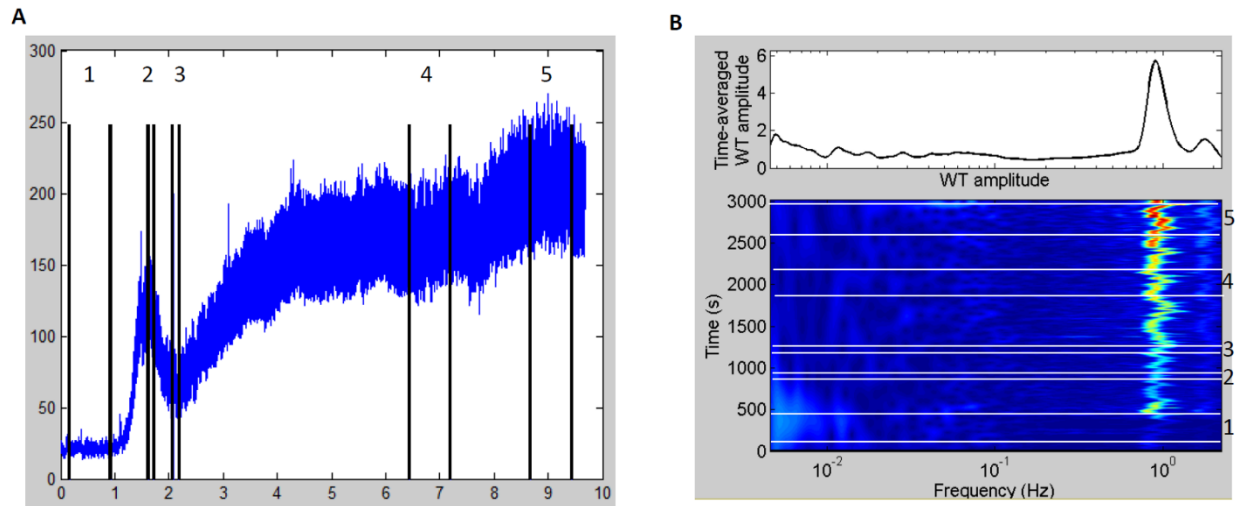


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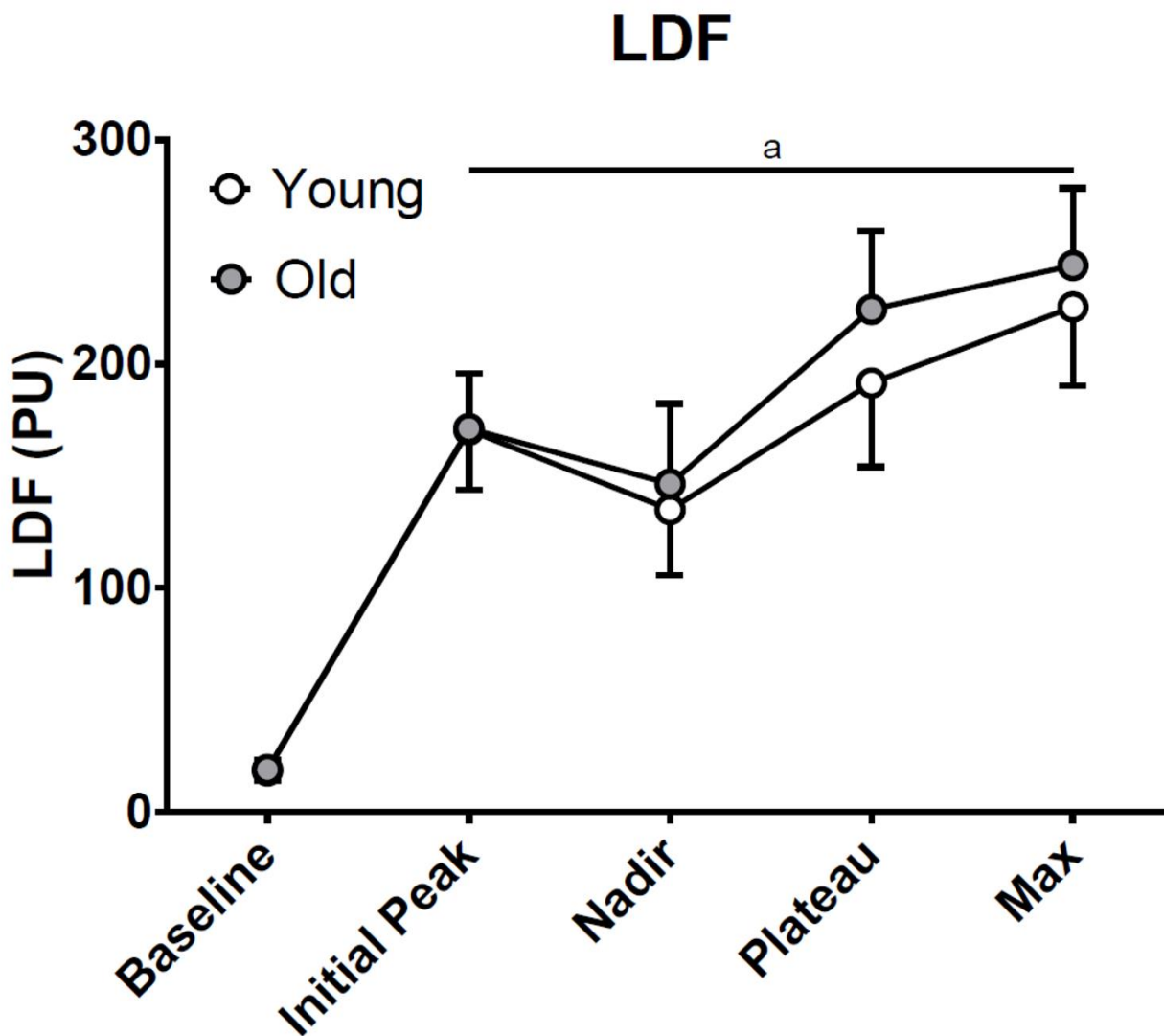
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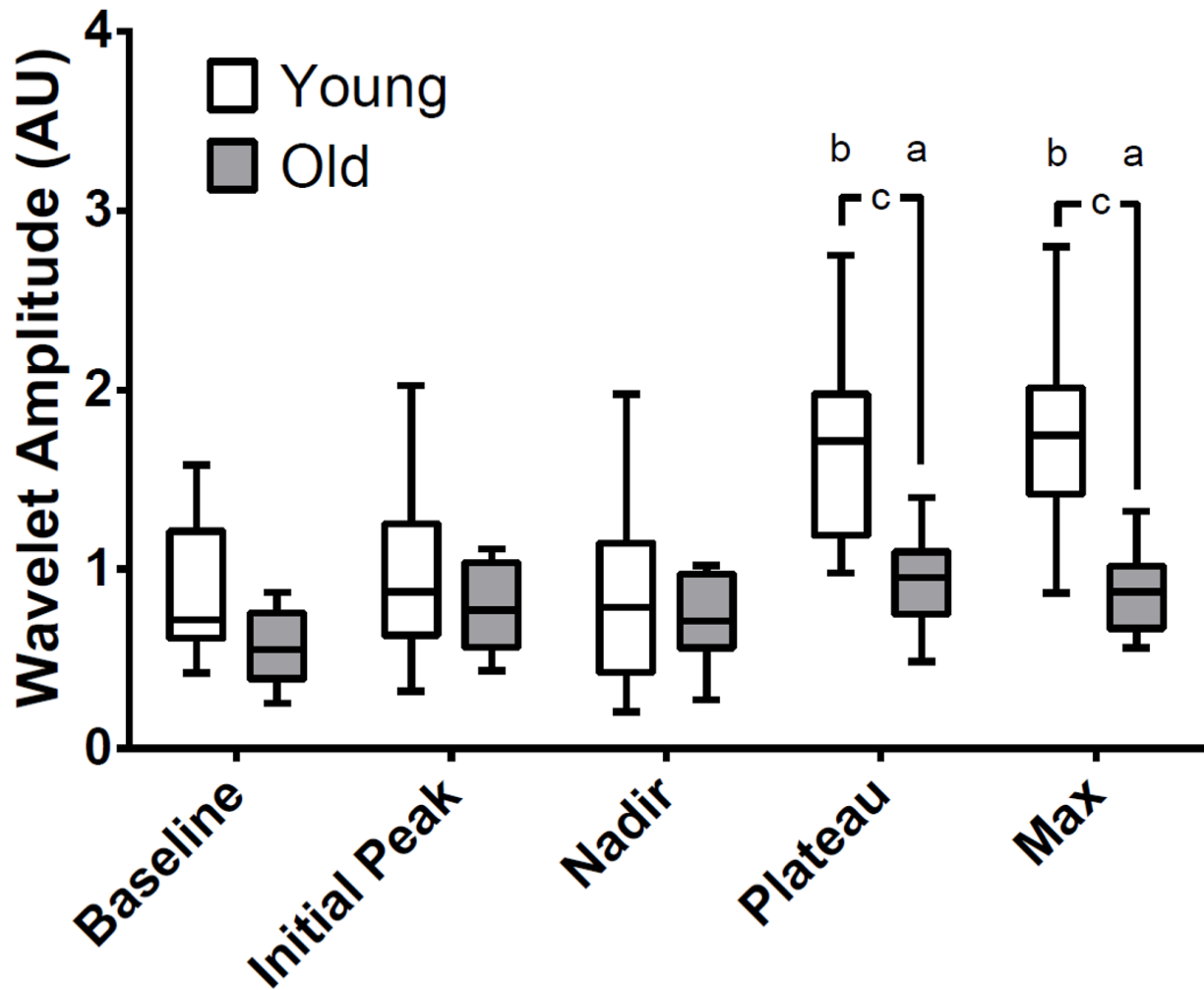
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**Figure 1. Data from a representative participant.** The laser-Doppler flux and sections selected for analysis (A) and the wavelet transform and corresponding sections for analysis (B). Each band, labelled 1 through 5, represents the time points used from the tracing to extract the data for baseline, initial peak, nadir, plateau, and max.

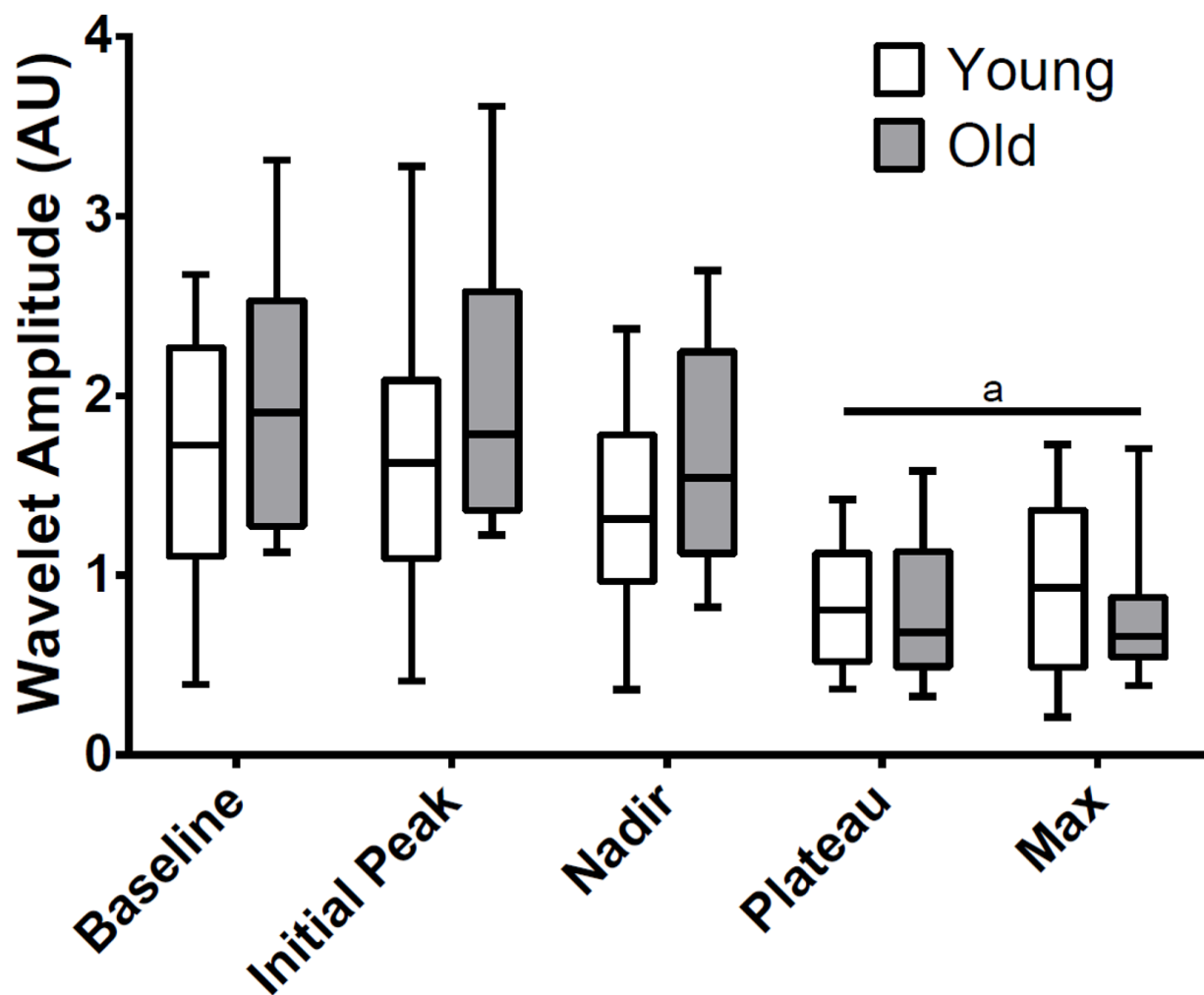


**Figure 2. Mean laser-Doppler flux data from both groups for each phase of the local heating protocol.** There was a significant increase in flux in both groups in response to local skin heating to 42 °C. There were no differences between the groups. a =  $P < 0.05$  compared to baseline.

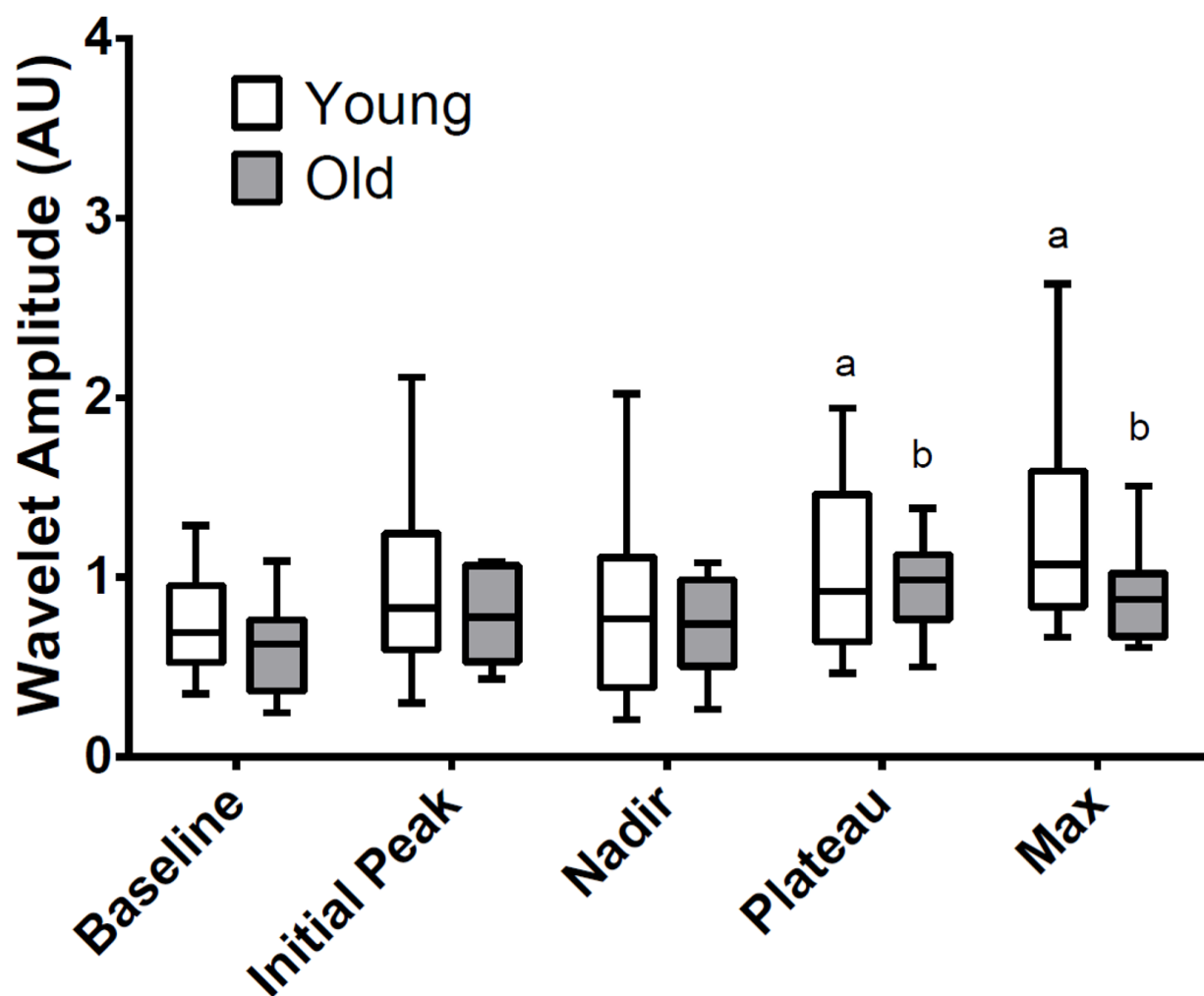


**Figure 3. Endothelial activity of both groups before and during local skin heating.**

Endothelial activity was increased in both groups following sustained local heating to 42 °C (plateau phase) and to 44 °C (max phase). The increase in endothelial activity was greater in the young group compared to the older group. Box plots display median, quartiles, min, and max. a =  $P < 0.05$  compared to baseline; b =  $P < 0.05$  compared to baseline, initial peak, and nadir; c =  $P < 0.05$  between groups.



**Figure 4. Neural activity of both groups in response to local skin heating.** Decreased neural activity was observed during the plateau and max phases in both groups. Box plots display median, quartiles, min, and max. a =  $P < 0.05$  compared to baseline, initial peak, and nadir.



**Figure 5. Myogenic activity in younger and older individuals in response to local skin heating.** Increases were observed in both groups, with the younger groups presented with increased myogenic activity in response to skin heating to 44 °C, while the older groups presented with increased activity in response to both 42 and 44 °C heating. Box plots display median, quartiles, min, and max. a =  $P < 0.05$  compared to baseline; b =  $P < 0.05$  compared to baseline and nadir.



## Tables

**Table 1.** Participant characteristics. Mean  $\pm$  standard deviation.

	<b>Young</b>	<b>Older</b>
<b>Age (years)</b>	24 $\pm$ 1	64 $\pm$ 1*
<b>Body Mass (kg)</b>	76 $\pm$ 6	80 $\pm$ 6
<b>Stature (cm)</b>	179 $\pm$ 2	178 $\pm$ 3
<b>Resting heart rate (beats<math>\cdot</math>min<math>^{-1}</math>)</b>	56 $\pm$ 2	52 $\pm$ 4
<b>Resting blood pressure (mm Hg)</b>		
<b>Systolic</b>	118 $\pm$ 4	122 $\pm$ 4
<b>Diastolic</b>	66 $\pm$ 4	76 $\pm$ 3*
<b><math>\dot{V}O_2\text{max}</math> (ml<math>\cdot</math>kg<math>^{-1}\cdot</math>min<math>^{-1}</math>)</b>	50 $\pm$ 5	36 $\pm$ 5*

\*indicates  $P < 0.05$  between young and older groups.